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PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of M. South et al.
Serial No. 09/716,962
Filed November 20, 2000
Confirmation No. 2205
Art Unit 1624
For SUBSTITUTED POLYCYCLIC ARYL AND HETEROARYL PYRIDONES USEFUL FOR SELECTIVE INHIBITION OF THE COAGULATION CASCADE
Examiner T. Truong

June 5, 2002

DECLARATION OF MICHAEL S. SOUTH UNDER 37 C.F.R. 1.132

I, Michael S. South, hereby declare as follows:

1. I am a resident of St. Louis, Missouri and am a named co-inventor of the matter described in Application Serial Number 09/716,962 entitled SUBSTITUTED POLYCYCLIC ARYL AND HETEROARYL PYRIDONES USEFUL FOR SELECTIVE INHIBITION OF THE COAGULATION CASCADE (the "Application").

2. I am currently employed by Pharmacia Corporation as a Science Fellow and have been so employed since April, 1983 in that capacity. Generally, my duties include medicinal chemistry. Attached is my *Curriculum Vitae* detailing my education and past work experience. .

3. Example 29 was disclosed in the Application on pages 222-224 as originally filed.

4. The compound of Example 29 was isolated and purified using the following procedure:

A catalytic amount of palladium on carbon (10%) in methanol was added to the pyridinone compound (0.20g, 0.32mmol) in methanol and the mixture was stirred under a balloon of hydrogen at room temperature for 12 hours. The mixture was filtered through celite and the solvent was evaporated to afford the product. The product was purified by reverse-phase chromatography to afford 0.24g (39%) of a brown solid.

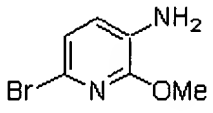
^{1}H NMR ppm (deuteriomethanol): 1.26 (d, 6H), 3.63 (septet, 1H), 4.44 (d, 2H), 4.65 (d, 2H), 6.27 (dd, 1H), 6.78 (dd, 1H), 7.62 (m, 9H);

HPLC purity (retention time): >99% (2.39 min);

HRMS calculated for $C_{24}H_{29}N_7O_2$ (M^+ + H) 448.2456, found 448.2447.

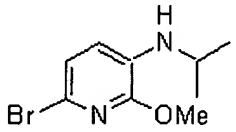
5. The compound of Example 30 was disclosed in the Application as originally filed on page 254, lines 4 and 5.

6. The compound of Example 30 was prepared according to the following process:



EX-30A

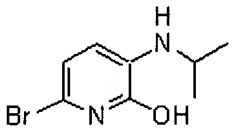
A solution of 2,6-dibromopyridine (10.4 g, 41.5 mmol) and sodium methoxide (15.7 g, 290 mmol) in dioxane was heated to reflux for 48 hours. The brown reaction was allowed to cool to room temperature and diluted with saturated solution of ammonium chloride. The solution was extracted with ether and the organic layer was washed with water, brine, dried over magnesium sulfate, and filtered. The solvent was removed by evaporation to afford the crude product. The product was purified by column chromatography (20% ethyl acetate-hexane) to afford 7.1 g (84%) of a red solid of product **EX-30A**; ^{1}H NMR ppm (deuteriochloroform): 3.94 (s, 3H), 6.71 (d, 1H, J = 6.0Hz), 6.83 (d, 1H, J = 6.0Hz); ^{13}C NMR ppm (deuteriochloroform): 67.2, 120.4, 122.5, 124.1, 130.3, 152.4; HPLC purity (retention time): 94% (2.92 min); HRMS calcd for $C_6H_7Br_1N_2O$ (M^+ + H) 204.9800, found 204.9769.



EX-30B

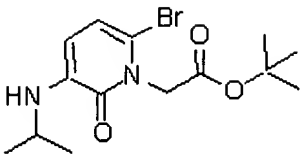
A solution of titanium tetrachloride 1M in dichloromethane (37 mL, 37 mmol) was added to a solution of **EX-30A** (6.8 g, 33.6 mmol) and acetone (3.3 mL, 44.9 mmol) in

dichloromethane. After stirring at room temperature for 3 hours, sodium cyanoborohydride (6.3 g, 100 mmol) was added and the solution stirred at room temperature for 14 hours. The solution was diluted with ether and water. The organic layer was washed with brine, dried over magnesium sulfate, and filtered. The solvent was removed by evaporation to afford the crude product. The product was purified by column chromatography (5% ethyl acetate-hexane) to afford 5.7 g (69%) of a red oil of product **EX-30B**; ^{1}H NMR ppm (deuteriochloroform): 1.20 (d, 6H), 3.51 (septet, 1H), 3.95 (s, 3H), 6.62 (d, 1H, J = 6.0Hz), 6.88 (d, 1H, J = 6.0Hz); HPLC purity (retention time): 95% (3.97 min); HRMS calcd for $C_9H_{13}Br_1N_2O$ (M^+ + H) 245.0289, found 245.0240.



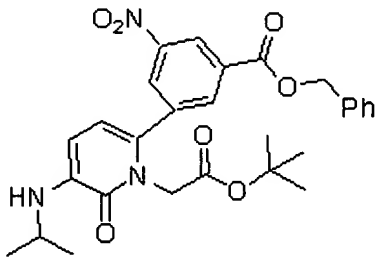
EX-30C

A solution of boron tribromide 1M in dichloromethane (46.0 mL, 46 mmol) was added to a solution of the methyl ether, **EX-30B,** (5.6 g, 0.23 mmol) in dichloromethane at -10°C. The solution warmed to room temperature and stirred for 16 hours. The reaction mixture was diluted with water, neutralized with a saturated sodium bicarbonate solution, and extracted with ether. The organic layer was washed with water, brine, dried over magnesium sulfate, and filtered. The solvent was removed by evaporation to afford the crude product. The product was purified by column chromatography (40% ethyl acetate-hexane) to afford a white solid of product **EX-30C**; ^{1}H NMR ppm (deuteriochloroform): 1.22 (d, 6H), 3.49 (septet, 1H), 6.20 (d, 1H), 6.88 (d, 1H); ^{13}C NMR ppm (deuteriochloroform): 22.3, 44.1, 110.0, 128.6, 132.2, 136.4, 160.0; HPLC purity (retention time): 94% (2.45 min); HRMS calcd for $C_8H_{11}Br_1N_2O$ (M^+ + H) 231.0133, found 231.0130.



EX-30D

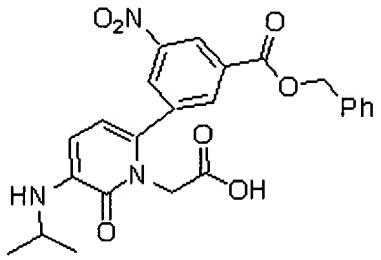
A suspension of CaH_2 (1.7 g, 40.3 mmol) in tetrahydrofuran was added to the pyridine, **EX-30C**, (4.2 g, 18 mmol) in tetrahydrofuran dropwise via an addition funnel. The resulting suspension was heated to reflux for 30 minutes. To the mixture was then added a solution of tert-butyl bromoacetate (2.9 mL, 19.6 mmol) in tetrahydrofuran (2.3 M). Refluxing of the mixture was continued for 18 hours. The reaction mixture was allowed to cool to room temperature, and quenched with an ice water mixture. The aqueous layer was extracted with ehter. The organic layer was washed with water, brine, dried over magnesium sulfate, and filtered. The solvent was removed by evaporation to afford the crude product. The product was purified by column chromatography (10% ethyl acetate-hexane) to afford 4.1 g (66%) of a white solid of product **EX-30D**; ^{1}H NMR ppm (deuteriochloroform): 1.08 (H20,H15) (d, 6H, J = 6.4Hz), 1.37 (H11,18,19) (s, 9H), 3.42 (H14) (septet, 1H, J = 6.4Hz, 8.4Hz), 4.81 (H7) (s, 2H), 5.05 (NH13) (d, 1H, J = 8.4Hz), 6.13 (H4) (d, 1H, J = 7.9Hz), 6.46 (H5) (d, 1H, J = 7.9Hz); ^{13}C NMR ppm (DMSO): 22.3 (C15,C20), 28.2 (C11,18,19), 43.6 (C14), 50.7 (C7), 82.5 (C10), 107.0 (C4), 111.8 (C5), 136.8 (C3), 158.4 (C2), 167.1 (C8); HPLC purity (retention time): 98% (4.63 min); HRMS calcd for $C_{14}H_{21}Br_1N_2O_3$ (M^+ + H) 345.0814, found 345.0827.



EX-30E

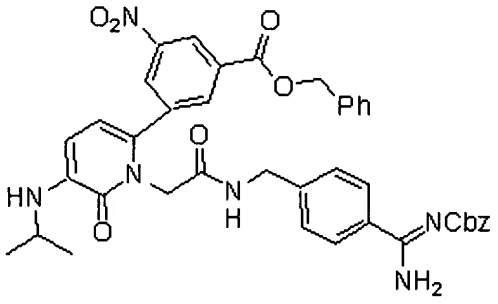
Tertakis(triphenylphosphine)palladium(0) (0.20 g, 0.17 mmol) was added to a mixture of the pyridone, **EX-30D**, (0.60 g, 1.7 mmol), boronic acid (0.68 g, 2.3 mmol), and cesium carbonate (2.2 g, 6.7 mmol) in 18 mL of anhydrous dioxane. The resulting

mixture was stirred at 80°C for 16 hours. The reaction mixture was allowed to cool to room temperature and was diluted with water and extracted with ether. The organic layer was washed with water, brine, dried over magnesium sulfate, and filtered. The solvent was removed by evaporation to afford the crude product. The product was purified by column chromatography (25% ethyl acetate-hexane) to afford 0.70 g (78%) of a red solid of product **EX-30E**; ^{1}H NMR ppm (deuteriochloroform): 1.24 (d, 6H,), 1.39 (s, 9H), 3.57 (septet, 1H), 4.41 (s, 2H), 5.39 (s, 2H), 6.14 (d, 1H), 6.40 (d, 1H), 7.40 (m, 6H), 8.35 (m, 1H), 8.42 (m, 1H), 8.85 (m, 1H); HPLC purity (retention time): >99% (5.70 min); HRMS calcd for $C_{28}H_{31}N_3O_7$ (M^+ + H) 522.2240, found 522.2242.



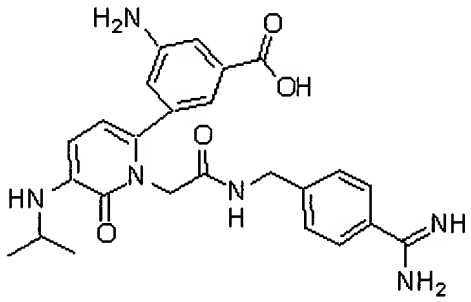
EX-30F

The t-butylester, **EX-30E**, (0.60g, 1.1 mmol) was dissolved into 4 N hydrochloric acid in dioxane and stirred at room temperature for 22 hours. The solvent was removed under a stream of nitrogen (NOTE! heating the acid results in decarboxylation to afford the N-methyl) to afford a red gum of product **EX-30F**; ^{1}H NMR ppm (deuteriochloroform): 1.53 (d, 6H,), 3.86 (septet, 1H), 4.55 (s, 2H), 5.39 (s, 2H), 6.42 (d, 1H), 7.39 (m, 5H), 8.29 (d, 1H), 8.37 (m, 1H), 8.44 (m, 1H), 8.94 (m, 1H); HRMS calcd for $C_{24}H_{23}N_3O_7$ (M^+ + H) 466.1614, found 466.1625.



EX-30G

PS-carbodiimide (2.3 g, 2.3 mmol) (1.00 mmol/g) was added to a slurry of the acid, **EX-30E**, (0.53 g, 1.1 mmol), 1-hydroxybenzotriazole (0.16 g, 0.71 mmol), benzamidine (0.44 g, 1.3 mmol), and N-methylmorpholine (1.3 mL, 11.8 mmol) in a dichloromethane-dimethylformamide (3:1) solution and the suspension was agitated for 13 hours. Upon completion of the reaction, the polyamine resin (2.81 mmol/g) (5.6 mmol) and polymer-bound aldehyde (2.3 mmol/g) (2.30 mmol) were added and the suspension was agitated for 1 hour. The solution was filtered and the polymer was rinsed with dimethylformamide and dichloromethane until no more UV activity was seen in the dichloromethane washing. The solvent was removed by evaporation to afford the crude product. The product was purified by reverse-phase chromatography to afford 0.73 g (87%) of an orange solid of product **EX-30G**; ^{1}H NMR ppm (deuterioacetone): 1.19 (d, 6H,), 3.48 (septet, 1H), 4.41 (d, 2H), 4.63 (s, 2H), 5.18 (s, 2H), 5.48 (s, 2H), 6.30 (m, 2H), 7.40 (m, 11H), 7.99 (d, 1H), 8.18 (bt, 1H), 8.58 (m, 1H), 8.68 (m, 1H), 8.78 (m, 1H); HPLC purity (retention time): 90% (4.50 min); HRMS calcd for $C_{40}H_{38}N_6O_8$ (M^+ + H) 731.2829, found 731.2853.



Example 30

A catalytic amount of palladium on carbon (10%) in methanol was added to the pyridinone compound, **EX-30G**, (0.33 g, 0.45 mmol) in methanol and the mixture was stirred under a balloon of hydrogen at room temperature for 6 hours. The mixture was filtered through celite and the solvent was evaporated to afford the product. The product was purified by reverse-phase chromatography to afford 0.24 g (39%) of a yellow solid of product **Example 30**; ^{1}H NMR ppm (deuteriomethanol): 1.26 (d, 6H), 3.63 (septet, 1H), 4.44 (d, 2H), 4.65 (d, 2H), 6.27 (dd, 1H), 6.78 (dd, 1H), 7.62 (m, 9H); HPLC purity (retention time): >99% (2.39 min); HRMS calcd for $C_{25}H_{28}N_6O_4$ (M^+ + H) 477.2250, found 477.2253.

7. The compounds of Example 29 and Example 30 have been tested for biological activity using the assays described on Application pages 273-275. The compounds were tested for activity against human tissue factor VIIa ("TF-VIIa"), human thrombin ("Thrombin"), human factor Xa ("Factor Xa") and trypsin type IX from porcine pancreas ("Trypsin"). The results of such testing are set forth below.

Example Number	**TF-VIIa IC_{50} (uM)**	**Thrombin IC_{50} (um)**	**Factor Xa IC_{50} (um)**	**Trypsin IC_{50} (um)**
29	0.052	>30	>30	0.044
30	0.118	>30	>30	0.049

8. Both Example 29 and 30 exhibited activity against TF-VIIa. Further, the compounds appear to be far more active for TF-VIIa than Thrombin and Factor Xa. In particular, compound 29 was at least 500 times more active against TF-VIIa than for Thrombin and Factor Xa, while compound 30 was as least 250 times more active against TF-VIIa than for Thrombin and Factor Xa.

9. Based on the above data for compound 29 and compound 30, these compounds offer the advantage of being selective for TF-VIIa over both Thrombin and Factor Xa.

10. I further declare that all statements made herein of my own knowledge are true and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. §1001, and that such willful and false statements may jeopardize the validity of the document or any patent issuing thereon.

Date: 6-05-2002 Michael S. South

Michael S. South